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CD8+ T Cells Rapidly Acquire NK1.1 and NK Cell-Associated Molecules Upon Stimulation In Vitro and In Vivo

Erika Assarsson,* Taku Kambayashi,* Johan K. Sandberg,* Seokmann Hong,† Masaru Taniguchi,‡ Luc Van Kaer,* Hans-Gustaf Ljunggren,* and Benedict J. Chambers2*

NK T cells express both NK cell-associated markers and TCR. Classically, these NK1.1+TCRαβ+ cells have been described as being either CD4+CD8− or CD4−CD8+. Most NK T cells interact with the nonclassical MHC class I molecule CD1 through a largely invariant Vα14-Jα281 TCR chain in conjunction with either a Vβ2-, -7, or -8 TCR chain. In the present study, we describe the presence of significant numbers of NK1.1+TCRαβ+ cells within lymphokine-activated killer cell cultures from wild-type C57BL/6, CD1d−/−, and Jα281−/− mice that lack classical NK T cells. Unlike classical NK T cells, 50–60% of these NK1.1+TCRαβ+ cells express CD8 and have a diverse TCR Vβ repertoire. Purified NK1.1+CD8α+ T cells from the spleens of B6 mice, upon stimulation with IL-2, IL-4, or IL-15 in vitro, rapidly acquire surface expression of NK1.1. Many NK1.1+CD8+ T cells had also acquired expression of Ly-49 receptors and other NK cell-associated molecules. The acquisition of NK1.1 expression on CD8+ T cells was a particular property of the IL-2Rα+ subpopulation of the CD8+ T cells. Efficient NK1.1 expression on CD8+ T cells required Lck but not Fyn. The induction of NK1.1 on CD8+ T cells was not just an in vitro phenomenon as we observed a 5-fold increase of NK1.1+CD8+ T cells in the lungs of influenza virus-infected mice. These data suggest that CD8+ T cells can acquire NK1.1 and other NK cell-associated molecules upon appropriate stimulation in vitro and in vivo. The Journal of Immunology, 2000, 165: 3673–3679.

The nonclassical MHC class I molecule CD1 is recognized by a specific subset of lymphocytes called NK T cells (1–4). NK T cells in C57BL/6 (B6) mice are defined as lymphocytes expressing TCR in conjunction with NK1.1 and another NK cell-associated molecules. These NK T cells are restricted in their TCR usage to an invariant Vα14-Jα281 chain associated with either a Vβ2, -7 or -8 chain (4–7). Mice deficient in CD1 or Jα281 are largely devoid of NK T cells, as a consequence of their dependence on the CD1 ligand and the invariant Vα14-Jα281 chain of the TCR (6–10).

NK-R1-P1 (CD161) is a family of disulfide-linked homodimers expressed on the surface of both NK and NKT cells (11). NKPR1A and C are activating receptors that can trigger both cytokine production and cytolytic activity by NK cells (12, 13), while NKPR1B appears to have inhibitory activity (14, 15). The Ab PK136 recognizes the NKPR1C receptor in the mouse, referred to as NK1.1. This Ab depletes NK cells in vivo (16, 17) and induces activation and proliferation of both NK and NKT cells in vitro (18, 19). NK and NKT cells in mice also express C-type lectins of the Ly-49 family (13, 20). Most Ly-49 molecules act as inhibitors for both cytokotoxicity and cytokine production (21–23) but at least two, Ly-49D and Ly-49H, activate NK cells (24, 25). Therefore, the activity of NK and NKT cells appears to be tightly controlled by a series of positive and negative signals.

In the course of the characterization of lymphokine-activated killer (LAK)3 cell cultures from NK T cell-deficient Jα281−/− and CD1d−/− mice, we found surprisingly that these cultures contained a substantial number of cells coexpressing TCRαβ and NK1.1. These cells did not resemble “classical” NK T cells, because a majority of them were CD8+ and expressed a broad spectrum of TCR Vβ-chains. In the present study, we characterize this subset of “nonclassical” NK T cells from B6, CD1d−/−, and Jα281−/− mice in vitro and in vivo.

Materials and Methods

Mice

B6 mice (6- to 10-wk-old) were obtained from the Microbiology and Tumor Biology Center of the Karolinska Institutet (Stockholm, Sweden). CD1d−/− (9), Jα281−/− (6), Lck−/− (26), Fyn−/− (27), and TAP2−/− (28) mice have been described previously. Mutant mice were backcrossed at least three times to a B6 background. All mice were maintained at the Microbiology and Tumor Biology Center of the Karolinska Institutet. Animal care was in accordance with national and institutional guidelines.

Abs and flow cytometry

All Abs were used according to the manufacturers’ recommendations. For flow cytometric analysis, cells were preincubated with 2.4G2 supernatant for 20 min to block Fc receptors. FITC-, PE- or biotin-labeled anti-2B4, -CD3e (145-2C11), -CD4 (KH-CD4), -CD8α (53-6.7), -CD8β2 (53-5.8), -CD69 (H1.2F3), -CD122 (TM-β1), -Ly-49A (A1), -Ly-49C1 (SE6), -Ly-49D (4E5), -Ly-49G2 (4D11), -NK1.1 (PK136), -αβ TCR (H57-597), -Vβ2 TCR (B20.6), -Vβ3 TCR (KJ-25), -Vβ4 TCR (KT4), -Vβ5.1, 5.2 TCR (MR9-4), -Vβ6 TCR (RR4-7), -Vβ7 TCR (TR310), -Vβ8.1, 8.2 TCR (MR5-2), -Vβ9 TCR (MR10-2), -Vβ10+ (B21.5), -Vβ11 (RR3-15), -Vβ12 TCR (MR11-1), -Vβ13 TCR (MR12-3), -Vβ14 (14-2), and -Vβ17a (KJ-23) were obtained from Pharmingen (San Diego, CA). Anti-Ly-49G2 (4D11) was purified from hybridoma culture supernatants (hybridoma obtained from American Type Culture Collection, Rockville, MD). Anti-Ly-49G2 (4D11) is identical to Lym-1 (4D11) and is specific for the αβ TCR. Anti-Ly-49G2 (4D11) is identical to Lym-1 (4D11) and is specific for the αβ TCR.

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3 Abbreviations used in this paper: LAK, lymphokine-activated killer; βm, β2-microglobulin; B6, C57BL/6; KIR, killer cell inhibitory receptor.
Type Culture Collection, Manassas, VA) on a protein G column (Amersham Pharmacia Biotech, Uppsala, Sweden). Streptavidin-FITC was obtained from Dakopatts (Glostrup, Denmark) and streptavidin-RED670 from Life Technologies (Rockville, MD). Cells were analyzed on a FACS-Scan or a FACSsort cytometer (Becton Dickinson, Mountain View, CA).

Generation of LAK cells

Single cell suspensions from spleens were depleted from erythrocytes and resuspended in complete medium (oMEM, 10 mM HEPES, 2 × 10⁻⁷ M 2-ME, 10% FCS, 100 U/ml penicillin, and 100 U/ml streptomycin) and human rIL-2 at the concentrations indicated (PeproTech, Rocky Hill, NJ), murine rIL-4, rIL-12, rGM-CSF (PeproTech), human rIL-7, and rIL-15 (R&D, Minneapolis, MN) were used at 10 ng/ml. Human rFN-α and murine rFN-α (PeproTech) were used at 1000 U/ml. Anti-CD3 Ab (145-2C11) was used at 10 μg/ml. Cells were cultured at 37°C in a humidified 10% CO₂ atmosphere in air for 5 days when not else noted. Cell sorting was performed using a FACSvantange cell sorter (Becton Dickinson).

CD8α⁺ cell purification

Two days before purification, B6 mice were depleted of NK cells by i.p. injection of 50–100 μg purified anti-NK1.1 Ab/mouse. The CD8α⁺ cells were isolated using the magnetic activated cell sorting (MACS) separation system (Miltenyi Biotec, Bergisch Gladbach, Germany), according to the manufacturer’s guidelines. Briefly, 1–2 × 10⁶ erythrocyte-depleted B6 splenocytes were washed once with buffer (PBS without Ca²⁺ and Mg²⁺) supplemented with 0.5% BSA and 2 mM EDTA. A total of 10 μl CD8α (Ly-2) microbeads and 90 μl buffer were added per 10⁶ cells and incubated at 8°C for 15 min and the cells were washed twice. CD8α⁺ cells were removed from the cell suspension using an LS⁺ column. Purity of cells was determined by flow cytometry. The MACS-separated cells were washed once and resuspended in complete medium plus 1000 U/ml IL-2 and plated in six-well plates (0.5–1 × 10⁶ cells/ml). Cells were cultured at 37°C in a humidified 10% CO₂ atmosphere in air.

For experiments examining IL-2Rβ (CD122)⁺ CD8⁺ T cells, purified CD8α⁺ cells were washed once and stained with IL-2Rβ-FITC or -PE and sorted by flow cytometry into IL-2Rβ⁺ CD8⁺ and IL-2Rβ⁻ CD8⁺ T cell populations. The cells were cultured in complete medium with 1000 U/ml IL-2 and analyzed for NK1.1 expression on day 3 and day 6.

RNA isolation, reverse transcription, and RT-PCR

Total cellular RNA was extracted using the Trizol RNA isolation method (Life Technologies), followed by cDNA synthesis of 2 μg RNA using the First-strand CDNA synthesis kit (Amersham Pharmacia Biotech) according to the protocol using pdN6 primers. PCR analysis was performed using DNA polymerase (Boehringer Mannheim, Mannheim, Germany) on a Programmable Thermal Controller PTC-100 (MJ Research, Watertown, MA) with 10-s denaturation at 94°C, 30-s annealing at 55°C, and 2-min extension at 72°C for 34 cycles. The primer sequence used for the reverse transcription reaction was for NKR-P1C-specific primers (14): 5’-GGTGAAGATGCACAGCAAGATAC and 3’-GAGTCACAGAAGGGAAGGCA (Cybergene, Stockholm, Sweden) encompassing nucleotides 96–1170 of murine NKR-P1C. CD8α gene quality was confirmed by amplification of a β-actin gene fragment using specific primers. Photo image of an ethidium bromide-stained gel is shown.

Preparation of cells from lungs of influenza-infected mice

Mice were infected intranasally with influenza A virus Japan/305/57 (a kind gift from Dr. A. D. Diehl, Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, Sweden). Ten days postinfection, both infected and control mice were sacrificed by cervical dislocation and their lungs were removed. The lungs were minced into small pieces and incubated in RPMI 1640 (10% FCS) with 250 U/ml collagenase type IV (Sigma, St. Louis, MO) at 37°C for 90 min. The erythrocytes were lysed and single cell suspensions were prepared from the digested lungs. Adherent cells were removed from the lung cellular suspension by incubating the cells on tissue culture petri dishes at 37°C for 90 min.

Results

Identification of NK1.1⁺ CD8⁺ T cells in LAK cell cultures from NKT cell-deficient mice

In LAK cell cultures from B6, NKT cell-deficient CD1d¹⁻⁻, and Jα281⁻⁻ mice, NK1.1⁺ T cells were detected after 5 days of culture in IL-2. The numbers of NK1.1⁺ T cells in LAK cell cultures from the NKT cell-deficient mice were similar to those from B6 mice (Fig. 1). Using three-color flow cytometry, 50–60% of these NK1.1⁺ T cells were found to express CD8α which is normally not expressed on classical NKT cells (Fig. 2). A majority of the CD8α⁺ T cells (70–90%) also coexpressed CD8β. The remaining CD8⁺ NK1.1⁺ T cells (referred to as NK1.1⁺ CD8⁺ T cells) revealed that they expressed a diverse TCR repertoire because they were polyclonal with regards to Vβ expression (Fig. 3). This observation, and the fact that these cells could be generated from NKT cell-deficient mice, suggested that these cells were not classical NKT cells.

CD8⁺ T cells are the precursors of NK1.1⁺ CD8⁺ T cells

Among fresh splenocytes, 1–3% of all CD8⁺ T cells expressed NK1.1. After treating mice with anti-NK1.1 Ab to remove NK1.1⁺ cells in vivo, NK1.1⁺ CD8⁺ T cells were no longer detected in the spleen cell population. To demonstrate that CD8⁺ T cells could acquire expression of NK1.1, CD8α⁺ cells were purified from mice pretreated with anti-NK1.1 Ab (Fig. 4A, left panel). When these CD8α⁺ cells were examined after 5 days of culture with IL-2, up to 40% of the cells had acquired expression of NK1.1 (Fig. 4A, right panel). To extend these observations, RT-PCR analyses for NKR-P1C (NK1.1) was performed using RNA from freshly isolated CD8⁺ cells from B6 mice pretreated with anti-NK1.1 Ab and similar CD8⁺ cells after culture with IL-2 for 5 days. Before culture, no NKR-P1C transcripts were detected in the CD8⁺ cells, whereas a marked increase in NKR-P1C transcripts was observed.

FIGURE 1. NK1.1⁺ TCRαβ⁺ cells are detected in LAK cell cultures from mice deficient in classical NKT cells. LAK cells from B6 (A), CD1d¹⁻⁻ (B), and Jα281⁻⁻ (C) mice were analyzed using three-color flow cytometry using anti-NK1.1-PE Ab, anti-CD8β-FITC Ab, and anti-TCRαβ-biotin Ab and streptavidin-RED670. The results are representative of four independent experiments.
NK1.1<sup>+</sup>CD8<sup>+</sup> T cell population. Taken together, all these observations are consistent with the notion that CD8<sup>+</sup> T cells in the LAK cell cultures can acquire expression of NK1.1, and that the transcriptional activation precedes expression at the cell surface.

**Dose responses and kinetics of NK1.1 expression**

Because the initial experiments were performed with relatively high concentrations of IL-2, we determined the minimal IL-2 concentration required for NK1.1 induction on CD8<sup>+</sup> T cells. NK1.1 expression was readily observed on CD8<sup>+</sup> T cells at a concentration of 10 U/ml (Fig. 5), and occasionally at levels as low as 1 U/ml (data not shown). There was also a gradual increase in the number of NK1.1<sup>+</sup>CD8<sup>+</sup> T cells when CD8α<sup>+</sup> cells were exposed to increasing concentrations of IL-2. At IL-2 concentrations between 10 and 1000 U/ml, we observed induction of NK1.1 expression already within the first 48 h of culture (Fig. 5).

**Acquisition of Ly-49 molecules**

Because up-regulation of NK1.1 was observed on IL-2-stimulated B6-derived CD8<sup>+</sup> T cells, we examined whether other NK cell-associated molecules such as the Ly-49 molecules were induced on these cells. Only a small percentage of purified CD8α<sup>+</sup> T cells from young B6 mice (<3 mo old) expressed Ly-49 molecules and the expression levels were generally low (Table I; data not shown). However, a significant proportion of NK1.1<sup>+</sup>CD8<sup>+</sup> T cells acquired expression of Ly-49A, Ly-49C/I, and Ly-49G2 after 72 h of culture with IL-2, whereas most NK1.1<sup>+</sup>CD8<sup>+</sup> T cells remained Ly-49 negative (Table I). This suggests that the acquisition of

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**Figure 2.** The majority of the NK1.1<sup>+</sup>TCRβ<sup>+</sup> cells within LAK cell cultures from B6, CD1d<sup>−/−</sup>, and Io281<sup>−/−</sup> mice express CD8. Expression of CD8α and NK1.1 was analyzed on the TCRβ<sup>+</sup> cells with three-color flow cytometry using anti-NK1.1-PE Ab, anti-CD8β-FITC Ab, and anti-TCRβ-biotin Ab/streptavidin-RED670 within the LAK cell cultures from B6 (A), CD1d<sup>−/−</sup> (B), Io281<sup>−/−</sup> (C), and TAP/β<sub>2</sub> (D) mice. TAP/β<sub>2</sub> mice have greatly reduced numbers of CD8<sup>+</sup> T cells, explaining the low numbers of NK1.1<sup>+</sup>CD8<sup>+</sup> T cells among the TCRβ<sup>+</sup> cells within LAK cells from these mice. The results are representative of four independent experiments.

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**Figure 3.** NK1.1<sup>+</sup>CD8<sup>+</sup> T cells are polyclonal with regards to Vβ expression. Three-color flow cytometry was performed using NK1.1-PE Ab, anti-CD8β-FITC Ab, and anti-Vβ-biotin/streptavidin RED670. All data are presented as percent (± SE) of cells expressing a given TCR Vβ.

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**Figure 4.** Expression of NK1.1 is induced after incubation of purified CD8<sup>+</sup> splenic T cells with IL-2. A. Analysis of NK1.1 and CD8β expression on freshly purified CD8α<sup>+</sup> splenic cells from B6 mice pretreated with anti-NK1.1 Ab (left panel) and after 5 days of culture in 1000 U/ml IL-2 (right panel). The CD8β<sup>−</sup> cells represent CD8α<sup>−</sup> cells. NK1.1 expression was detected on up to 40% of all CD8<sup>+</sup> T cells (both CD8α<sup>+</sup> and CD8αβ<sup>+</sup>). The results are representative of at least eight independent experiments. B. RT-PCR analysis of RNA from freshly purified CD8α<sup>+</sup> splenic cells from B6 mice pretreated with anti-NK1.1 Ab (lane 1) and after 5 days of culture in 1000 U/ml IL-2 (lane 2). cDNA quality was confirmed by using β-actin primers (lanes 3 and 4). The results are representative of three independent experiments. The arrows represent the migration of size marker fragments.
NK1.1 occurs in parallel with the acquisition of the inhibitory Ly-49 molecules, Ly-49 A, C/I and G2. We also examined expression of the activating molecule Ly-49D on NK1.1 cells whereas 20% of normal LAK cell-derived NK cells expressed Ly-49D (data not shown).

Cytokines that induce NK1.1 expression on CD8α+ T cells

The ability of IL-2 to induce expression of NK1.1 on CD8α+ T cells may not be a unique property of this cytokine. Thus, other cytokines were examined with respect to their ability to induce NK1.1 expression on CD8α+ T cells. CD8α+ T cells cultured with IL-4 or IL-15 for 5 days were found to express NK1.1 at levels similar to cells cultured with IL-2 (Fig. 6, A–C), whereas cells cultured with IL-7, IL-12, GM-CSF, IFN-α, IFN-γ, or anti-CD3 Ab did not (Fig. 6D; data not shown). This observation suggests that IL-2, IL-4, and IL-15 up-regulated NK1.1 on the cell surface of CD8α+ T cells through a common pathway such as via the common γ-chain.

NK1.1+CD8α+ T cells arise from IL-2β+CD8α+ T cells

Although sorted CD8α+ T cells were found to acquire expression of NK1.1 after cytokine stimulation, it was not entirely clear whether this was a function of all CD8α+ T cells or a subpopulation of CD8α+ T cells. Because ~10–20% of freshly derived CD8α+ T cells expressed IL-2β (data not shown), CD8α+ T cells were sorted by flow cytometry into IL-2β+ and IL-2β− populations and cultured in IL-2. When the cells were examined after 6 days of culture, ~35% of the IL-2β+CD8α+ T cells coexpressed NK1.1 (Fig. 7A), whereas only 3% of the IL-2β−CD8α+ T cells were NK1.1+ (Fig. 7B). These observations suggest that expression of IL-2β was a prerequisite for IL-2-induced NK1.1 expression on CD8α+ T cells.

Efficient NK1.1 expression on CD8α+ T cells requires Lck but not Fyn

IL-2β can transduce signals through soluble protein kinases such as Lck and Fyn. When LAK cells were prepared from Fyn−/− mice, the number of NK1.1+CD8α+ T cells generated was equivalent to the number found in B6 LAK cell cultures. However, LAK cell cultures from Lck−/− mice had a significantly reduced number of NK1.1+CD8α+ T cells compared with B6 LAK cell cultures (data not shown). Because this reduced number of NK1.1+CD8α+ T cells could be related to the low frequency of T cells in Lck−/− mice, purified CD8α+ T cells from Lck−/− mice were cultured with IL-2 to determine whether Lck signaling was required for up-regulation of NK1.1 expression. Even though ~50% of the purified CD8α+ T cells from the Lck−/− mice expressed IL-2β (data not shown), only 8% of the purified CD8α+ T cells from

### Table I. Expression of Ly-49 molecules on NK1.1+CD8α+ and NK1.1+CD8α− cells

<table>
<thead>
<tr>
<th>Ly-49</th>
<th>% CD8α+</th>
<th>% NK1.1+CD8α+</th>
<th>% NK1.1+CD8α−</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 3</td>
<td>Day 6</td>
</tr>
<tr>
<td>A</td>
<td>1.2 ± 0.7</td>
<td>5.9 ± 2.4</td>
<td>5.7 ± 1.9</td>
</tr>
<tr>
<td>C/I</td>
<td>1.5 ± 0.3</td>
<td>2.8 ± 1.0</td>
<td>2.6 ± 1.0</td>
</tr>
<tr>
<td>G2</td>
<td>1.6 ± 0.4</td>
<td>11.0 ± 5.0</td>
<td>9.2 ± 2.9</td>
</tr>
</tbody>
</table>

*CD8α+ T cells were purified from B6 mice pretreated with anti-NK1.1 Ab and cultured in IL-2. Cells were analyzed for Ly-49 expression by flow cytometry. Data were collected from at least three different experiments and are expressed as percentage ± SE of cells expressing a given Ly-49 molecule.
NK1.1 can be up-regulated on CD8\(^+\) cells coexpressed NK1.1 (Fig. 9). This observation suggests that Lck is required for efficient IL-2-mediated expression of NK1.1 on CD8\(^+\) T cells.

**Virus infection can up-regulate NK1.1 expression on CD8\(^+\) T cells**

The fact that CD8\(^+\) T cells could up-regulate NK1.1 and other NK cell-associated molecules upon cytokine stimulation in vitro led us to address whether this could occur in vivo. NK1.1 was up-regulated on ~1–3% of CD8\(^+\) T cells in the lungs of untreated B6 mice (Fig. 8). However, 10 days after infection with influenza A virus Japan/305/57, elevated numbers of NK1.1+CD8\(^+\) T cells were detected in the lungs. At this time point, ~10% of the CD8\(\alpha\)β\(^+\) T cells coexpressed NK1.1 (Fig. 9B). This observation suggests that NK1.1 can be up-regulated on CD8\(^+\) T cells in vivo.

**Discussion**

In the present study, we demonstrate that a subpopulation of CD8\(^+\) T cells acquire expression of NK1.1 and other NK cell-associated molecules in vitro upon stimulation with cytokines and in vivo during viral infection. Cell surface expression of NK1.1 on CD8\(^+\) T cells could be detected within the first 48–72 h of culture in IL-2, IL-4, or IL-15. Unlike the classical CD1-restricted NKT cells which normally express only V\(\beta\)2, -7, or -8 (3, 4), these NK1.1+CD8\(^+\) T cells displayed a diverse TCR V\(\beta\) repertoire similar to the pattern observed for splenic CD8\(^+\) T cells. The expression of NK1.1 was transcriptionally controlled, as NKR-P1C message in CD8\(^+\) T cells could only be detected following IL-2-stimulation. The potential to acquire NK1.1 expression was found to be a specific property of IL-2R\(\beta\)/CD8\(^+\) T cells. The protein kinase Lck appeared to be critical in triggering efficient NK1.1 expression on CD8\(^+\) T cells, because purified CD8\(^+\) T cells from Lck\(\sim\) mice did not express NK1.1 following culture in IL-2. Thus, a subpopulation of CD8\(^+\) T cells rapidly acquire expression of NK1.1 and other NK cell-associated molecules under appropriate stimulation in vitro.

NK1.1+CD8\(^+\) T cells were also found in the lungs of mice infected with influenza A virus. These cells were detected at low numbers in the lungs of uninfected mice, but represented up to 10% of the total CD8\(^+\) population at day 10 postinfection. The majority of the NK1.1+CD8\(^+\) T cells were CD8\(\alpha\)β\(^+\)TCR\(\alpha\)β\(^+\) and also expressed the NK cell-associated Ly-49 receptors. Interestingly, up to 20% of these NK1.1+CD8\(^+\) T cells were virus-specific as determined by MHC class I tetramer staining. As observed with the IL-2 derived NK1.1+CD8\(^+\) T cells, these cells were distinct from conventional NKT cells because they were also found in influenza-infected CD1\(^+\) mice (29).

The definition of NKT cells is not entirely clear. Classically, NKT cells are defined by their coexpression of TCR and NK cell-associated markers such as NK1.1. Most NKT cells have been characterized as being either CD4+ CD8\(^-\) or CD4\(^-\)CD8\(^+\), and express a restricted TCR repertoire that is specific for CD1 (1–4). However, not all NKT cells are CD1-restricted and some have been reported to express CD8. NK1.1+CD8\(\alpha\)\(^+\) T cells have been found previously in LAK cell cultures from B6 mice (30, 31). In one of these studies, NK1.1+CD8\(\alpha\)\(^+\) T cells were described to exist in IL-4-derived LAK cell cultures but not in IL-2-derived LAK cell cultures (30). NK1.1+CD8\(^+\) T cells have also been reported to be a minor subset of NKT cells in vivo (32–37). In a study on lymphocytic choriomeningitis virus-infected mice, a marked increase in the numbers of NK1.1+CD8\(^+\) T cells was observed (37). Whether these NK1.1+CD8\(^+\) T cells are CD1-restricted is not clear as one study found that their numbers did not change in CD1\(^+\) mice (32), while another found a dramatic reduction in the number of NK1.1+CD8\(^+\) T cells from the bone marrow of CD1\(^+\) mice (35). It has also been suggested that NK1.1+CD8\(^+\) T cells are a distinct lineage as they can arise even in thymectomized mice (33). Coles et al. (36) have demonstrated that Ly-49\(^+\)NK1.1+CD8\(^+\) T cells are prevalent in older mice. In their study, it was made clear that these cells represented a subpopulation of normal T cells rather than “aberrant” NKT cells. Our results differ from some of the observations described above but...
are in line with others. In contrast to the study of Ballas and Rasmussen (30), we found that both CD8αα+ and CD8αβ+ T cells could acquire NK1.1. Furthermore, NK1.1+CD8+ T cells were detected in LAK cell cultures from both CD1d1−/− and Jα281−/− mice in equal number to those observed in B6 mice. Our data suggest that a significant proportion of the NK1.1+CD8+ T cells observed in the present study arise from the IL-2Rβ+/CD8+ T cells following activation with IL-2 and during influenza infection (29). Thus, NK1.1+CD8+ T cells most likely represent a state of activation or differentiation of CD8+ T cells rather than the selective expansion of a distinct cell lineage. The expression of CD4 and CD69 on a majority of the NK1.1+CD8+ T cells is in line with this notion. These cells differ from classical NKT cells in a number of aspects. First, they could be detected even in CD1d1−/− and Jα281−/− mice that are deficient in classical NKT cells. Second, the NK1.1+CD8+ T cells are polyclonal with regards to TCR Vβ expression and finally, they do not appear to recognize CD1 (our unpublished observations).

Expression of NK cell-associated molecules on T cells may be induced by cytokines triggering the expression of proteins encoded within the NK gene complex. The NK gene complex has been described in both rodents and humans. In mice, the complex is found on chromosome 6 whereas in humans it is located on chromosome 12 (38). The NK gene complex contains the genes for NKR-P1, CD69, and the killer cell inhibitory receptors (KIRs), Ly-49 (in mouse) and CD94 (in mouse and human). Because only cytokines that use the common γ-chain appeared to up-regulate NK1.1 on the T cells, one may speculate that signaling through this chain could activate specific genes within the NK gene complex. Support for this hypothesis comes from the observation that CD94/NKG2A could be up-regulated on human CD8+ T cells treated with IL-15 (39). Interestingly, the up-regulation of the CD94/NKG2A complex was associated with inhibition of the cytotoxic capability of these T cells (39, 40). Other studies with human CD8+ T cells have demonstrated that stimulation with IL-12 up-regulated expression of NKR-P1A, whereas IL-2 and IL-15 down-regulated NKR-P1A (41). It should be noted that NKR-P1A may have different functions than NKR-P1C (NK1.1) and so could be regulated in a different manner to accommodate its functions. Similarly, the lack of Ly-49D on the NK1.1+CD8+ T cells may indicate that the expression of this molecule is regulated differently than that of the other Ly-49 molecules examined.

The protein kinases Lck and Fyn are critical in the development of T cells and NKT cells in vivo (26, 27). Lck−/− mice have reduced numbers of CD4+CD8− T cells and single-positive cells in the thymus and consequently reduced numbers of peripheral T cells. Fyn−/− mice have normal T cell development but two recent studies have found that the number of NKT cells is reduced in these mice (42, 43). It has been demonstrated that Fyn binds to CD3ζ, and Lck to CD4 and CD8αc; however, both Lck and Fyn can bind the IL-2Rβ (44, 45). LAK cells generated from Fyn−/− mice had equivalent numbers of NK1.1+CD8+ T cells to those derived from B6 mice. In contrast, LAK cells generated from Lck−/− mice had significantly lower numbers of NK1.1+CD8+ T cells. Furthermore, purified CD8+ T cells from Lck−/− mice could not up-regulate NK1.1 in the presence of IL-2 despite the fact that 50% of these cells expressed IL-2Rβ compared with 10–20% in B6 mice (our unpublished observations). Therefore, lack of Lck signaling could be related to the diminished ability of the IL-2Rβ+/CD8+ T cells in the Lck−/− mice to express NK1.1.

Non-MHC-restricted killing is an in vitro phenomenon that is observed in long-term cultured T cells and in T cell cultures stimulated with high concentrations of IL-2 (46). To date, a molecular explanation for this phenomenon has yet to be elucidated. One may speculate that the up-regulation of NK cell-associated molecules allows T cells to expand their killing capabilities to include innate non-MHC-restricted killing. Indeed, we have observed that some CD8+ T cell lines expressing NK1.1 targeted not only specific peptide-loaded cells but also the prototypic NK cell target, YAC-1, whereas CD8+ NK1.1− T cell lines did not kill YAC-1 cells (our unpublished observations). However, because there is no known ligand for NK1.1 on YAC-1 cells, YAC-1 killing might also relate to the expression of other unknown activating molecules on these T cells. Although non-MHC-restricted killing may be an in vitro phenomenon, such killing could possibly also play a critical role in controlling the spread of infections in vivo.

The induction of Ly-49 molecules on CD8+ T cells may be of importance in regulating the fate of activated CD8+ T cells. With respect to human CD8+ T cells, it has been speculated that the induction of KIR expression of human CD8+ T cells may be by their inhibitory functions dampen T cell activity and so prevent the CD8+ T cells from becoming “exhausted” by overstimulation. The latter could render them a selective advantage during memory cell development (36, 47). Several other studies with human CD8+ T cells also support a notion where expression of KIR or CD94/NKG2 receptors may regulate T cell function (39, 40, 48). The latter may also be the case for inhibitory Ly-49 molecules. Indeed, studies using mice transgenic for Ly-49 molecules have found that CD8+ T cell function is affected in these mice (49, 52). In relation to Ly-49 molecules, it is of interest to note that the significant population of memory CD8+ T cells in the mouse express Ly-49 receptors (36).

NK cells have become appreciated for their potential roles in a variety of infectious diseases (53, 54) as well as in host responses against some tumors (55). Much of this knowledge has been derived in mice depleted of NK cells using anti-NK1.1 Abs. With the present observation that some CD8+ T cells can acquire expression of NK1.1, there may now be a requirement for the reanalysis of some of the data that have implicated a role for NK cells in host responses to infections and tumors. Indeed, the appearance of NK1.1+CD8+ T cells in the lungs of influenza-infected mice and lymphocytic choriomeningitis virus-infected mice (37) suggests that these cells do appear in vivo upon infection and do not merely represent an in vitro artifact. In particular, T cell responses to influenza were reported to be low in mice treated with anti-NK1.1 Ab (56). One may speculate that in this case, viral-specific T cells had been removed by anti-NK1.1 Ab administration that could explain the unresponsiveness.

In conclusion, the present data describe the induction of expression of NK1.1 and other NK cell-associated molecules on a subpopulation of CD8+ T cells. These data suggest that CD8+ T cells can acquire NK cell-associated receptors upon appropriate stimulation in vitro and in vivo.

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